IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Brian D. FOLLSTAD	Docket No.:	3374-A
Serial No.:	10/620,064	Group Art Unit No.:	1651
Filed:	July 15, 2003	Examiner:	Leon Lankford, Jr.
For:	METHODS AND MEDIA FOR CONTROLLING SIALYLATION OF PROTEINS PRODUCED BY MAMMALIAN CELLS		

DECLARATION OF DR. CAROLE HEATH UNDER 37 CFR § 1.132

Commissioner for Patents
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I, Carole Heath, do hereby declare as follows that:

1. I, Carole Heath, am currently a Director of Process Development in charge of the Department of Cell Science and Technology at Amgen, Inc. I have been employed in the area of mammalian cell culture development for the purpose of protein production at Immunex Corporation and then at Amgen Corporation, which acquired Immunex in 2002, from 1998 to the present. My Curriculum Vitae is attached.

2. In my opinion, one of skill in the art of designing cell culture processes for production of proteins in July of 2002 would have at least about four years of relevant experience in process development for commercial production of proteins or in a post-graduate academic setting. In many, perhaps most, cases, it could take longer to achieve skill in the art of designing cell culture processes. One of skill in this art would likely have at least Bachelor's or Master's degree, but would be more likely to have a Ph.D. This degree could be in any of a variety of related disciplines such as biology, biochemistry, chemical engineering, or molecular or cellular biology. Such a person would be able to read, understand, and evaluate scientific publications and apply this knowledge to the design of processes. One of skill in the art would have knowledge of

and would appreciate basic principles of scientific analysis, including the meaning and importance of a "control" sample, as compared to an "experimental" sample.

3. One of skill in the art would understand that an experimental control is that part of an experiment that allows one to determine which factor varied in the experiment has an impact on the experimental outcome. An experimental sample may vary only one factor or may vary multiple factors relative to a control sample. If only one factor is varied, that factor would be considered to be influencing experimental outcome. If multiple factors are varied, the experiment must be carefully designed so as to make it possible to draw conclusions about any one factor. Such "multifactorial" experiments must be carefully designed so as to make it possible to draw meaningful conclusions. *See e.g.* Ghannoum et al. 91995), Antimicrobial Agents and Chemotheraphy 39(11): 2459-65. Multifactorial design is used frequently in the field of cell culture due to the numerous variables to be handled in designing cell culture processes. If more that one factor is varied in the absence of a multifactorial design, it would be difficult, if not impossible, to attribute any experimental outcome to a particular factor.

4. I have read US Patent No. 6,673,575 (hereinafter, Franze).

5. Example 5 of Franze describes the cultivation of two cell cultures, which were compared with respect to the distribution of isoforms of erythropoietin produced by the cultures, as follows:

> Nutrient solution was continuously supplied to the culture during the growth phase. For this purpose a storage vessel containing nutrient solution was coupled to the fermenter. The nutrient solution contained amino acids, vitamins, insulin, trace elements, salts, glutamine and carbohydrates. Two fermentations were carried out as follows:
>
> In fermentation A the nutrient solution contained D-(+)-glucose as the sugar and in fermentation B the sugars were D-(+)-glucose, D-(+)-galactose and D-(+)-mannose. The mass ratio of glutamine to the sugars was 1:2.2:3.6:6 in fermentation B. The concentration of the individual sugars in the nutrient solution was between 7.2 and 18 g/l.
>
> The glutamine concentration in the culture was periodically analysed in fermentation B and the consumption was calculated. The momentary volume flow of the nutrient solution was matched to the requirement of the cells for nutrients. In fermentation A the glutamine concentration was not used as a controlled variable. The nutrient solution in fermentation B contained a mixture of the sugars D-(+)glucose, D-(+)galactose and D-(+)mannose in a mass ratio of 2:3:5. The concentration of all sugars in the fermenter was kept in the range 2 to 6 g/l during the culture by corresponding feeding.

Franze, col. 8, lines 42-65.

One of skill in the art would appreciate that this description gives no clear information on several issues that could be important for interpreting the experiment.

The glucose concentration of fermentation A is not mentioned. The phrase "[t]he concentration of all sugars in the fermenter was kept in the range 2 to 6 g/l" leaves uncertainty as to whether each sugar in fermentation B is at a concentration within the stated range or the concentration all three sugars together is within the stated range. The volume of nutrient solution added to fermentations A and B is not mentioned. This could potentially be important because the nutrient solution contains many components, any one or more of which could affect glycosylation, and because the volume of nutrient solution could affect concentrations of all medium components. The composition of the starting medium is not stated.

Thus, the results of the experiments could be reasonably attributed by one of skill in the art to one or more of the following factors: (1) different glucose concentrations in fermentation A and B; (2) different total concentrations of sugar in fermentations A and B; (3) differing volumes of nutrient solution fed to fermentations A and B; and (4) different sugars fed to fermentations A and B. Hence, one of skill in the art would be left with no unambiguous conclusion as to which experimental factor is responsible for the results of Example 5.

In Example 7 of Franze, the experiment performed is described in part as follows:

> The major difference between fermentations E and F is the addition of various monosaccharides to the feed medium.
> Fermentation E:
> The usual sugar D-(+)-glucose was used for fermentation E. The initial concentration was 3 g/l. By appropriately feeding the glucose-containing nutrient solution, the glucose concentration in the culture broth was maintained at $3^{\pm}0.5$ g/l during the entire culture.
> The culture period was typically $100^{\pm}20$ h. The concentration of EPO was typically $40^{\pm}10$ mg/l at the time of harvest.
> Fermentation F:
> In addition to D-(+)-glucose, the sugars D-(+)-galactose and D-(+)-mannose were added in a mass ratio of ca. 1:2:3 to the feed medium for fermentation F. During the culture the concentration of all sugars was kept in a range between 0.25 g/l and 3.5 g/l by appropriate feeding.
> The culture period was typically $100^{\pm}20$ hours. The concentration of EPO at the time of harvest was typically $40^{\pm}10$ mg/l.

Franze, col. 10, lines 33-52.

Information important for interpreting this experiment is missing from this description. The starting glucose concentration of fermentation F is not stated. Further, it is unclear whether (1) glucose, mannose, and galactose are each maintained in the

range of 0.5 g/l to 3.5 g/l in fermentation F or (2) the concentrations of glucose, mannose, and galactose together are maintained in the range of 0.5 g/l to 3.5 g/l in fermentation F. The volumes of nutrient solution added are not stated. Hence, one of skill in the art would conclude that the outcome of the experiment described in Example 7 could be attributed to one or more of the following factors: (1) different glucose concentrations; (2) different total concentrations of sugar; (3) different sugars; and/or (4) different volumes of nutrient solution fed.

6. A first aspect of the invention of Franze is described as follows: "the invention concerns a process for isolating a glycosylated polypeptide from eukaryotic cells, wherein the eukaryotic cells are cultured in a suitable medium and the desired polypeptide is isolated from the cells or/and the culture supernatant wherein the process is characterized in that a mixture of at least 2 and preferably at least 3 carbohydrates is added to the culture medium." Franze, col. 2-3, lines 64-3. A list of carbohydrates that can be used in the practice of this invention is provided, including "monosaccharides and disaccharides such as glucose, glucosamine, ribose, fructose, galactose, mannose, sucrose, lactose, mannose-1-phosphate, mannose-1-sulfate and mannose-6-sulfate." Column 3, lines 4-8. In another aspect, the process "is characterized in that nutrients are added in a controlled manner and according to requirements during the culture which comprise at least one essential amino acid for the respective cultured cell line or/and at least one carbohydrate. . ." The claims of Franze are more narrowly directed to processes "for increasing the glycosylation of a polypeptide when producing the glycosylated polypeptides in mammalian or insect cells." Franze, claims 1 and 4.

One of skill in the art would not expect to be able to use all monosaccharides and disaccharides, or even the eleven sugars named above, interchangeably in a cell culture process. Some, but certainly not all, monosaccharides and disaccharides, are involved in biosynthetic pathways that culminate in protein glycosylation. Those sugars that are involved in such pathways occupy different parts of the pathways and interact with different enzymes. "An enzyme usually catalyzes a single chemical reaction or a set of closely related reactions. The degree of specificity for substrate is usually high and sometimes virtually absolute." Stryer, Biochemistry, W.H. Freeman and Co., San Francisco, 1981, p. 104. Thus, different sugars are likely to interact with different

enzymes. Different enzymes may have different forward and reverse reaction rates, may have different concentrations of substrates or end products available, may be present at different concentrations and in different intracellular locations, may require other molecules in addition to the sugar to complete a step in the pathway, may or may not be subject to feedback inhibition, to name just a few of the many possible differences. Hence, one of skill in the art would not expect all monosaccharides and disaccharides to have similar effects on protein glycosylation. Published work is consistent with these expectations. As an example, Stark and Heath (Archives of Biochem. Biophys. 192(2): 599-609 (1979); submitted in an enclosed Information Disclosure Statement) found that at "concentration ranges up to 100 μg/ml, glucose and mannose stimulated glycosylation and [^{35}S]methionine incorporation whereas fructose, galactose, ribose, N-acetylglucosamine, glycerol, and pyruvate were without effect." Stark and Heath, at 605. Thus, based on the teaching of Franze and the knowledge in the art, one of skill in the art could not reasonably believe that adding any two or more monosaccharides or disaccharides to a cell culture would necessarily increase glycosylation.

7. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. § 1001), and may jeopardize the validity of the application or any patent issuing thereon.

12/13/06
Date

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JOB HISTORY

2006-	**Director**, Cell Science & Technology, Amgen Inc.
2004-2006	**Associate Director**, Cell Science & Technology, Amgen Inc.
2002-2004	**Scientist IV**, Amgen Inc. (Immunex acquired by Amgen in 2002)
2000-2002	**Senior Staff Scientist**, Immunex Corp.
1998-2000	**Staff Scientist**, Immunex Corp., Seattle, WA
1998-2001	**Adjunct Associate Professor**, Department of Chemical Engineering, Iowa State University, Ames, IA
1993-1998	**Associate Professor (with tenure)**, Department of Chemical Engineering, Iowa State University **Associate Professor**, Biomedical Engineering Program, Iowa State University **Associate Professor**, Neuroscience Program, Iowa State University
1988-1993	**Assistant Professor**, Thayer School of Engineering, Dartmouth College, Hanover, NH
1984-1988	**Graduate Research Assistant**, Rensselaer Polytechnic Institute, Troy, NY
1983-1984	**Instructor**, Department of Anesthesiology, School of Nursing, Albany Medical College, Albany, NY

EDUCATION

Ph.D.	**Chemical Engineering**, August 1988, Rensselaer Polytechnic Institute, Troy, NY
M.S.	**Chemical Engineering**, May 1988, Rensselaer Polytechnic Institute
M.S.	**Biomedical Engineering**, May 1984, Rensselaer Polytechnic Institute
B.S.	**Chemical Engineering**, May 1980, University of Rochester, Rochester, NY
B.A.	**Biology**, May 1978, University of Rochester

PROFESSIONAL HONORS

1994:	Newcomb Lecturer, Renowned Women in Chemical Engineering Seminar Series, Tulane University
1991-1996:	NSF Presidential Young Investigator Award
1989:	General Electric Young Faculty Fellowship

OTHER EXPERIENCE

2008:	Conference Co-Chair: Cell Culture Engineering XI, Australia
2006:	Session Co-Chair: Cell Culture Process Development - Advances in Process Engineering, ACS National Meeting, San Francisco, CA
2006:	Workshop Chair, Cell Culture Engineering X, Whistler, BC
2004:	Session Co-Chair: Update on Regulatory Issues for Cell Culture Engineers," Cell Culture Engineering IX, Cancun, Mexico
2002:	Organizing Committee Member, Cell Culture Engineering VIII, Aspen, CO
2000:	Session Co-Chair: Cultured Cells and Tissues for Transplantation and as Model Systems, Engineering VII Engineering Foundation Conference, Santa Fe, NM
1998:	Session Co-Chair: Advances in Tissue Engineering, AIChE National Meeting, Miami Beach, FL
1998:	Session Co-Chair: Tissue Engineering and Gene and Cell Therapy, ACS National Meeting, Boston, MA
1998:	Session Co-Chair: Quantification Approaches in Large Scale Culture, Cell Culture, Engineering VI Engineering Foundation Conference, San Diego, CA
1997-98:	Section Editor, Current Opinion in Biotechnology, Biochemical Engineering
1997:	Session Co-Chair: Tissue Engineering, ACS National Meeting, San Francisco, CA
1997-98:	Membership Committee, Sigma Xi, ISU Chapter
1996-98:	Director: Food, Pharmaceutical & Bioengineering Division, AIChE
1994-00:	Organizing Committee Member: Cell Culture Engineering V, VI, VII Engineering Foundation Conferences, San Diego, CA
1995:	Session Co-Chair: Tissue Engineering, AIChE National Meeting, Miami, FL
1994-96:	Scientific Advisory Panel Member: North Carolina Biotechnology Center, Academic Research Initiation Grants Program
1994:	Organizer and Session Chair: Science, Technology, & Gender Symposium, National Women's Studies Association National Meeting, Ames, IA
1994:	Session Co-Chair: Membrane Bioseparations, ACS National Meeting, San Diego, CA
1993:	Session Co-Chair: Bone and Tendon, 1st International Conference on Cellular Engineering, Stoke-on-Trent, England
1992-93:	Vice-President: Society of Women Engineers, North Country Chapter
1992:	Session Chair: Measurement of Intracellular and Cell-Surface Properties Session Co-Chair: Immobilized and Perfused Eucaryotic Cell Cultures, AIChE National Meeting, Miami Beach, FL
1989-93:	Co-Chair, Association for Women in Science, Dartmouth Chapter
1989:	Session Co-chair: Biopolymer Purification Session Co-chair: General Paper Session, MBTD, ACS National Meeting, Miami Beach, FL

PATENTS

Mallapragada, S., Heath C., Shanks, H., and Miller, C., Patterned substrates and methods for nerve regeneration, U.S. Patent No. 6,676,675, issued Jan. 13, 2004

PRESENTATIONS

Heath, C.A., Advancing our cell culture platform: Incorporating lessons learned and new technologies, AAPS Workshop on Current Trends in Monoclonal Antibody Development and Manufacturing, Boston, MA, June 2006

Heath, C.A. and Seewoester, T., Streamlining process development: Evolution of a cell culture platform for monoclonal antibodies, Cell Culture Engineering X, Whistler, BC, April 2006

Heath, C.A., Platform approach for cell culture processing of monoclonal antibodies, IIR Conference on Biopharmaceutical Production, San Francisco, CA, July 2005

Heath, C.A., Increasing production of Enbrel: Pathways to approval and beyond, 3rd International Symposium on Downstream Processing of Genetically Engineered Antibodies and Related Molecules, Nice, France, October 2004

Heath, C.A., Improving large-scale processes: From early definition to post-approval changes, IBC, San Diego, CA, December 2004

Heath, C.A. and Kiss, R., Process transfer, scale-up and comparability for a co-developed therapeutic antibody, IBC Cell Culture and Upstream Processing, Boston, MA, October 2004

Kiss, R. and Heath, C.A., Process transfer, scale-up, and comparability for a co-developed therapeutic antibody, Cell Culture Engineering IX, Cancun, Mexico, March 2004

Heath, C.A., Improving the production process for Enbrel® post-approval, Cell Culture Engineering IX, Cancun, Mexico, March 2004

PUBLICATIONS

Heath, C. and Kiss, R., Upstream processing: Cell culture process development: Advances in process engineering - Bioreactor and operational development, *Biotechnology Progress,* accepted (2006).

Rutkowski, G.E. and Heath, C.A., Development of a bioartificial nerve graft. I. Design based on a reaction-diffusion model, *Biotechnology Progress* 18 (2), 362-372 (2002).

Rutkowski, G.E. and Heath, C.A., Development of a bioartificial nerve graft. II. Nerve regeneration in vitro, *Biotechnology Progress,* 18 (2), 373-379 (2002).

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Heath, C. and Belfort, G., Immobilization of suspended mammalian cells: Analysis of hollow fiber and microcapsule bioreactors, *Adv. Biochem. Eng./Biotech.* 34, 1-31 (1987).

Heath, C.A., Lai, L., Bizios, R. and Malik, A.B., Pulmonary hemodynamic effects of antisheep serum-induced leukopenia, *J. Leukocyte Biol.* 39, 385-397 (1986).